

Physiological and Biochemical Neuroprotection in Cetaceans: Are Some Marine Mammal Species Safeguarded from Emboli Formation and Barotrauma?

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LONG-TERM GOALS

The primary goal of this study is to evaluate the susceptibility of critical tissues in cetaceans to acoustically mediated trauma from emboli formation. By investigating tissue and whole animal mechanisms we intend to identify possible physiological/environmental factors that would allow for lipid/gas mobilization and concomitant tissue damage at depth. If successful, the results of this project will enable the development of environmentally sensitive schedules for oceanic acoustic activities by identifying those species most susceptible to tissue injury.

OBJECTIVES

To accomplish these goals we are focusing on two key questions:

1. ***Are the neural tissues of marine mammals uniquely hypoxia tolerant due to the presence of neuroglobin and/or cytoglobin?*** This is being examined by measuring the concentration and function of oxygen-carrying globin proteins (hemoglobin, cytoglobin and neuroglobin) in the brain (both sensory and cognitive areas) of a wide variety of terrestrial, swimming and deep diving mammals including the beaked whales.
2. ***Is the dive response that safeguards marine mammals from decompression illness compromised by high levels of locomotor activity?*** In this part of the study we are measuring cardiovascular, metabolic, and gas transfer dynamics of trained bottlenose dolphins during sedentary and active periods underwater.

Together these studies will enable us to determine if some marine mammal species, such as the family of beaked whales, are more susceptible to non-auditory tissue damage as may occur in conjunction with navy and oil exploration sound operations. We will take into account several recent hypotheses regarding emboli formation as well as observed behavioral responses of marine mammals to low- and mid- frequency sound production.

APPROACH

This study uses two approaches to determine the relative susceptibility of cetaceans to acoustically mediated trauma. Because stranded marine mammals often display behaviors associated with neural dysfunction (i.e. disorientation, poor localization and righting responses), and neural tissues are exceptionally vulnerable to decompression damage, we are evaluating natural variation in

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neuroprotective mechanisms of the central nervous system. One team is conducting laboratory studies at the tissue level to assess the presence and function of oxygen binding globin proteins in the brain of marine mammals. Team members include specialists in morphology and pathology of marine mammals (M. Miller, CA Department of Fish and Game; D.A. Pabst, University of North Carolina-Wilmington), globin chemists (D. Kliger and R. Goldbeck, UCSC), molecular biologists (M. Zavanelli, UCSC) and physiologists (T.M. Williams and D. Casper, UCSC). Together we are conducting one of the first studies to examine neuroglobin and its potential role as a neuroprotectant in marine mammals. Using a comparative analysis of tissues collected from marine and terrestrial mammals we are determining the effects of dive capacity on the expression of globin proteins, and therefore the vulnerability of different species to hypoxia associated with decompression syndromes.

The second team involved in this study is examining the susceptibility of cetaceans to decompression illness at the whole animal/physiological level by monitoring disruption of bradycardia during diving tests. Tests with bottlenose dolphins trained to dive at varying depths and during different exercise states are assessing the variability in bradycardia and peripheral vasoconstriction as occurs with the dive response. The effects of a major physiological mechanism known to alter blood flow, exercise, is being investigated. We hypothesize that intense levels of exercise has the capacity to alter the dive response, and therefore, nitrogen transfer dynamics between tissues. In this study we are examining this by measuring heart rate, and post-dive blood parameters in trained dolphins under three conditions, 1) shallow diving in warm water, 2) moderate depth, warm water dives varying in exercise intensity, and 3) deep dives. Team members for this part of the program include physiologists (T.M. Williams, UCSC) and animal behaviorists (T. Fink and B. Richter, UCSC; P. Berry, EPCOT)

WORK COMPLETED

Tissue Globin Analyses. Our team has successfully developed two assays for brain globins, a spectrophotometric test that provides total globin concentration and an mRNA expression test for relative cytoglobin and neuroglobin levels. Currently, we have used these assays to detect the presence and concentration of globins in the cerebral cortex of 16 species of mammals. This includes five species of terrestrial mammal ranging in body mass from 0.1 kg to 100 kg, and 11 species of marine mammal ranging in mass from 30 to 300 kg. Among the marine species, we have examined both coastal and pelagic divers among the small cetaceans, pinnipeds and sea otters. All have demonstrated the presence of globins, although the concentration varies among the various species. Both the cerebral cortex and cerebellum have yielded similar results for the species in which we were able to sample both areas.

We are continuing to refine our isolation techniques in order to quantify the level of globins as well as characterize the exact molecular structure of the globins. One of the major challenges of this project has been obtaining fresh brain samples for the analysis. Although the globins appear robust we find that the morphological structure of neural tissue quickly degrades during postmortem events. As a result, identification of specific areas of globin concentration becomes difficult.

Variation in Diving Bradycardia. The second component of this study examines variability in the dive response of cetaceans. A major challenge has been developing heart signal instrumentation that could withstand the rapid swimming movements of dolphins. During this year we were able to solve this problem. We succeeded in developing, testing and collecting data from two custom built instrumentation systems for monitoring variability in the bradycardia response of freely-diving

dolphins. The first uses a medical Holter monitor designed for ambulatory human patients. Initial tests successfully recorded the ECG of two bottlenose dolphins in our facility. This system and the associated software enables detailed analysis of each heart beat for the animals. Consequently, we are able to obtain a detailed profile of the electrocardiogram of the dolphins as well as identify subtle and gross changes in the signals as occurs from rest to high levels of activity. The second instrument incorporates a 2-axis accelerometer that is used to correlate the level of activity (from stroke mechanics and angle of descent) with changes in heart rate in diving dolphins. Both instruments and associated underwater housings as well as custom fitted wetsuits for carrying the instrumentation in freely diving animals have been tested on diving dolphins during shallow (3 m) and moderate (10 m) depth performance. Variability in heart rate has been monitored during four physiological states at two depths, 1) resting on the water surface, 2) resting while submerged to depth, 3) routine swimming at depth, and 4) during rapid ascents. These tests are continuing and will soon include an investigation of dolphins diving in waters exceeding 25 m.

RESULTS

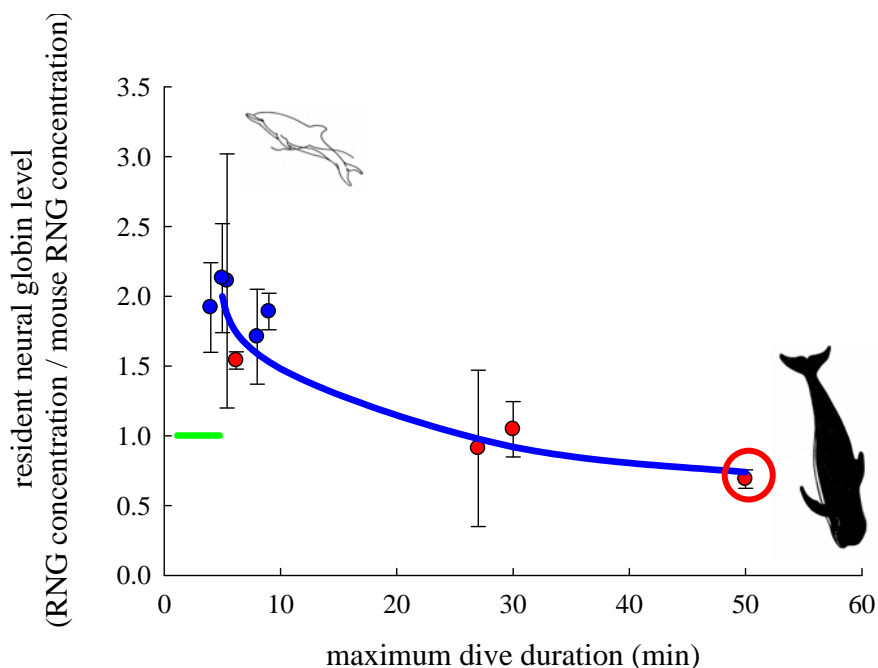


Figure 1. Resident neural globin (RNG) level in relation to maximum breath-hold duration in mammals. Circles and lines represent means ± 1 S.E.M for swimming (blue) and diving (red) specialists. The blue line is the least squares curvilinear regression as described in Williams *et al*, 2008; the green horizontal line in the lower left corner shows mean RNG and breath-hold capability for terrestrial mammals. The red circle denotes data for the Blainville's beaked whale which showed the lowest levels of RNG and longest maximum dive duration for the species examined.

During this year one of our primary accomplishments was the publication of the first comparative paper discussing neuroprotective globins in the mammalian brain (Williams *et al.*, 2008). Using biochemical and molecular assays for 16 species of terrestrial and marine mammals we found marked

variation in the potential of the mammalian brain to protect itself from hypoxic-ischemic injury through circulating (hemoglobin) and resident (neuroglobin, cytoglobin) globin proteins. In addition to significant differences between terrestrial and marine mammals, swimming and diving specialists showed characteristic differences in the relative level of globin proteins within the cerebral cortex. Interestingly, these levels correlated with the maximum diving duration recorded for each species in the wild (Fig. 1). The highest resident globins levels occurred in swimming specialists that tend to dive for less than 10 minutes. Conversely, the level of resident globins declined asymptotically for diving specialists among marine mammals (e.g. those species showing maximum dive durations ≥ 10 minutes). Although only one beaked whale was examined, this animal represented the extreme, with the lowest globin levels and longest dive durations.

The second major accomplishment concerned the monitoring of heart rate during different levels of exercise in the diving bottlenose dolphin. With the development of an ECG-accelerometer microprocessor we have been able to correlate discrete changes in heart rate with propulsive stroking during low and high speed swimming (Fig. 2). Initial tests indicate considerable variation in the level of bradycardia maintained by submerged dolphins, which depended on the activity state. For example, the heart rate adult bottlenose dolphins ranged from approximately 30 to 120 beats.min⁻¹. The highest and lowest heart rates exhibited by the dolphins occur while stationing on the water surface or while stationing on a submerged target, respectively. Submerged activity resulted in considerable variability in the level of bradycardia maintained in the diving animals, progressively increasing from sedentary stationing to slowly swimming at preferred speeds to rapid maneuvers such as quick turns or ascents. Further analysis is currently taking place to determine the effects of sequential diving on this cardiovascular variability.



Figure 2. Typical recording from the UFI electrocardiograph/accelerometer microprocessor used to simultaneously monitor changes in heart rate (top panel), swimming movements (middle panel) and dive angle (lower panel) in diving dolphins. The upper trace shows the typical mammalian ECG waveform. The lower two panels show the actual corresponding accelerometer output in red and the calculated mirror image in blue for assessing forward acceleration due to stroking and diving angle.

Together these preliminary results indicate that neuroprotection in diving marine mammals may be a species-specific balance between intrinsic and extrinsic factors. A suite of oxygen-binding globins appear to provide complimentary mechanisms for facilitating oxygen transfer into neural tissues as well as the potential for protection against reactive oxygen and nitrogen groups when marine mammals are submerged. A variable cardiovascular response when submerged enables the animals to meet the demands of exercise but raises a question regarding the movement of gases (oxygen, carbon dioxide and nitrogen) during diving. The vulnerability of essential tissues to injury during submergence will be addressed following further tests and the development of a model that incorporates the variability in tissue profiles and physiological responses observed in these studies.

IMPACT/APPLICATIONS

Our recent findings on variability in the cardiovascular response to diving and in tissue globin levels in the cerebral cortex provide:

- 1. A new perspective on neuroprotection.** By examining a wide variety of mammalian species living in different habitats, we demonstrate how malleable the mammalian brain can be when placed under extreme chronic hypoxia, which occurs not only in air-breathing vertebrates who dive but also in response to various common medical conditions in humans and other species.
- 2. An assessment of the importance of globin proteins.** Since neuroglobin and cytoglobin have been associated with neuronal survival following stroke and other ischemic insults with cardiovascular accidents, the results are relevant to many of the leading causes of mortality in the United States. Furthermore, although further research is needed, differences in resident neuroglobins may help to explain the relative susceptibility of deeper diving species to barotrauma following exposure to anthropogenic noise.
- 3. New techniques for clinical, ecological, behavioral and physiological studies.** Our study is the first to measure the concentration of resident neural globins by developing new biochemical methods and animal models. To our knowledge this is the first time that these globins have been investigated for any non-laboratory mammalian species, and provides new techniques for use by a wide variety of comparative and medical neurophysiologists. In addition, the instrumentation developed for monitoring cardiovascular changes in freely-diving marine mammals provides a new tool for assessing the response of wild mammals to anthropogenic disturbance.

RELATED PROJECTS

None.

PUBLICATIONS

Williams, T.M. and Zavanelli, M. (2007) Natural Neuroprotection in the Brains of Marine Mammals: Why swimming dolphins don't stroke. **Integrative and Comparative Biology** 47 (1), e1 [published, refereed].

Williams, T.M., Zavanelli, M., Miller, M.A., Goldbeck, R.A., Morledge, M., Casper, D., Pabst, D.A., McLellan, W., Cantin, L.P., and Kliger, D.S. (2008) Running, swimming and diving modifies neuroprotecting globins in the mammalian brain. **Proceedings Royal Society of London** 275, 751-758 [published, refereed].

HONORS/AWARDS/PRIZES

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